COMPARATIVE SENSITIVITY OF SPERM AND EGGS TO ULTRAVIOLET RADIATIONS

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The sperm of the sea urchin are more sensitive to ultraviolet radiations than the eggs when the effectiveness of the rays is compared by the retardation of cleavage of unexposed eggs fertilized with irradiated sperm on the one hand and of irradiated eggs fertilized with unexposed sperm on the other (Giese, 1939c). It would be interesting to know whether sperm are generally more susceptible to these radiations than eggs; therefore, the experiments were repeated on a number of marine forms. It is also desirable to find an explanation for this differential susceptibility in those cases where it occurs. Insight might be gained by determining action spectra for the sperm and egg, therefore the relative efficiency of action of different wave-lengths of ultraviolet light in retarding cleavage of irradiated eggs and of eggs fertilized with irradiated sperm was determined as described below.

Materials and Methods

Arbacia punctulata, Nereis limbata, Chaetopterus pergamentaceus, and *Mactra* sp. were studied at Woods Hole, Mass. *Strongylocentrotus franciscanus* and *S. purpuratus,* collected at Moss Beach, and *Urechis caupo* collected at Bolinas Bay, California, were used at Stanford University, and *Dendraster excentricus* and *Pateria miniata* were studied at the Hopkins Marine Station, each type of egg being used during the active breeding season.

The methods for studying the eggs were similar to those previously described (Giese, 1938). Except for the work on the action spectrum, the mercury argon discharge tube which emits about 85 per cent of its light at λ 2537 Å was used and the light intensity was measured with a Hanovia Ultraviolet Meter (No. 949). The dishes were kept in running sea water to attain a lower temperature than that of the room. The work on the action spectrum was done with a mercury arc and a natural quartz monochromator and the intensity of the light was measured with a thermopile as in previous studies (Giese, 1938). The eggs were kept in dishes in moist chambers and in a constant temperature room at 15° C.

Sperm were used in dilutions of between 1 : 200 and 1 : 1,000 of the spawn. Such dilution is necessary because in denser suspensions ultraviolet light is completely removed by the sperm first reached. Irradiated sperm lose their fertilizing power rapidly, therefore they must be used soon after exposure (see Hinrichs, 1927, for studies on inactivation).

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Experimental

Comparison of various eggs

A summary of the general results obtained with all the eggs studied will be found in Table I. Not all the eggs respond to ultraviolet radiations in the same way. Thus cleavage of eggs of Arbacia and Strongylocentrotus is merely slowed up but remains normal after small and medium dosages so that comparisons of the effects of various dosages and wave-lengths is relatively easy. Abnormalities only appear after larger dosages. In Urechis, Nereis, and some of the other eggs the threshold for abnormal development is relatively low. While per cent of abnormal development could be used for analysis of effects of radiations, it would be much more difficult.

It is readily apparent that with regard to ultraviolet susceptibility, there are two types of sperm: in the Echinoderms, especially Arbacia and Strongylocentrotus, the

Table I

Comparative action of ultraviolet radiation[2] on eggs and sperm of various marine animals

Species	Effects on eggs	Effects on sperm
Strongylocentrotus purpuratus	Delay just noticeable after about 100 ergs/mm.^{2}; will develop even after 4,000; after 8,000 ergs/mm.^{2} become quite abnormal.	Noticeable delay[3] even after 10–20 ergs/mm.^{2} Marked retardation as dosage above this is used.
Arbacia punctulata	Noticeable delay after 200 ergs/mm.^{2} but even after 2,000 ergs/mm.^{2} plutei, normal but smaller than controls, develop from eggs. After 4,000–8,000 ergs/mm.^{2} eggs are quite abnormal.	Noticeable delay even after less than 50 ergs/mm.^{2} After 250 ergs still develop larvae but after 500 quite abnormal. Even after 4,000 ergs/mm.^{2} sperm activate eggs.
Dendraster excentricus	Slight delay after 1,600 ergs/mm.^{2}; strong after 6,400; quite abnormal after 25,000 ergs/mm.^{2}	Slight delay after 200 ergs/mm.^{2}; abnormal after 800 ergs/mm^2.
Urechis caupo	Some delay after 200 ergs/mm.^{2} Marked injury with abnormal cleavage after 5,000 ergs/mm.^{2}	Marked abnormalities after 200 ergs/mm.^{2}
Chaetopterus pergamentaceus	Slight delay only after about 4,000 ergs/mm.^{2}; after 16,000 ergs/mm.^{2} still cleave but much delay and many cytolize.	Slight delay after 2,000 ergs/mm.^{2}; killed after about 8,000–16,000 ergs/mm^2.
Nereis limbata	Even after 4,000 ergs/mm.^{2} develop with little delay to the trochophore stage; after 8,000 ergs show delayed cleavage.	Slight delay between 4,000–8,000 ergs/mm.^{2} 8,000 kills most sperm.
Mactra sp.	Very slight delay after 500 ergs/mm.^{2}; striking after 4,000–8,000.	Delay after 250 ergs/mm.^{2} and progressive delay thereafter.

[2] The measurements with the Hanovia meter are accurate to about 10 per cent as checked by thermopile measurements in one instance.

[3] Amounting to 15–30 minutes delay at the third cleavage of eggs fertilized with such irradiated sperm. Marked delay means a delay of an hour or more.

sperm are much more sensitive than the eggs; in the worms such as Urechis, Nereis, and Chaetopterus the sperm is slightly if at all more sensitive than the egg, as judged by cleavage delay.

Such a lack of differences in susceptibility of the gametes might be more apparent than real. It is possible that when there is little or no cleavage delay following fertilization of an egg with an irradiated sperm, the sperm may be serving only to activate the egg to haploid parthenogenesis. Eggs of Arbacia and of Chaetopterus were, therefore, fertilized with sperm treated either to a small dosage or to a medium dosage of radiations and at appropriate intervals samples were fixed in Bouin's fluid and stained with iron hematoxylin. Although the preparations were

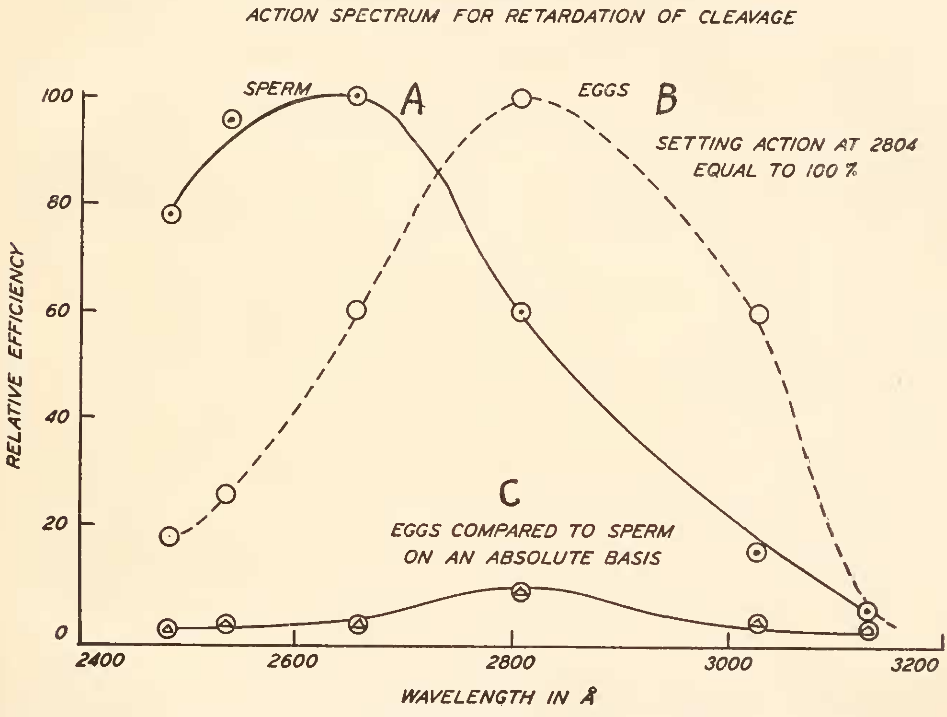

FIGURE 1. Action spectra for retardation of cleavage of eggs fertilized with irradiated sperm at *A* and for irradiated eggs at *C*. At *B* the data for the eggs are compared on a relative basis setting the value at λ 2,804 Å as 100 per cent efficient. See text below.

not entirely satisfactory, evidence for pronuclear fusion was observed in both cases. No lagging or disintegrating sperm were observed in the cytoplasm of either egg. Since neither cytological nor physiological evidence suggests parthenogenesis, it seems likely that for the dosage ranges tested the delayed cleavage follows fusion of the gametic nuclei. The difference between the two types of sperm must lie in some other factor. Possible explanations will be considered in the discussion.

The data in Table I show that the threshold for effects on cleavage is quite different for eggs of different species. Thus Strongylocentrotus, Arbacia, Mactra, and Urechis eggs are retarded after brief exposures to ultraviolet as compared to

Nereis, Chaetopterus, and Dendraster. Whether this is due to mere physical screening by some inert materials in the egg or to differences in concentration of some light sensitive materials is not known.

Action spectra for egg and sperm

If irradiation of the nucleus alone causes retardation of division of the cell, the same action spectrum should be found for egg and sperm; that is, there should be no qualitative difference in effectiveness of different wave-lengths even though the general susceptibility of the sperm is greater. If elements in the cell other than the

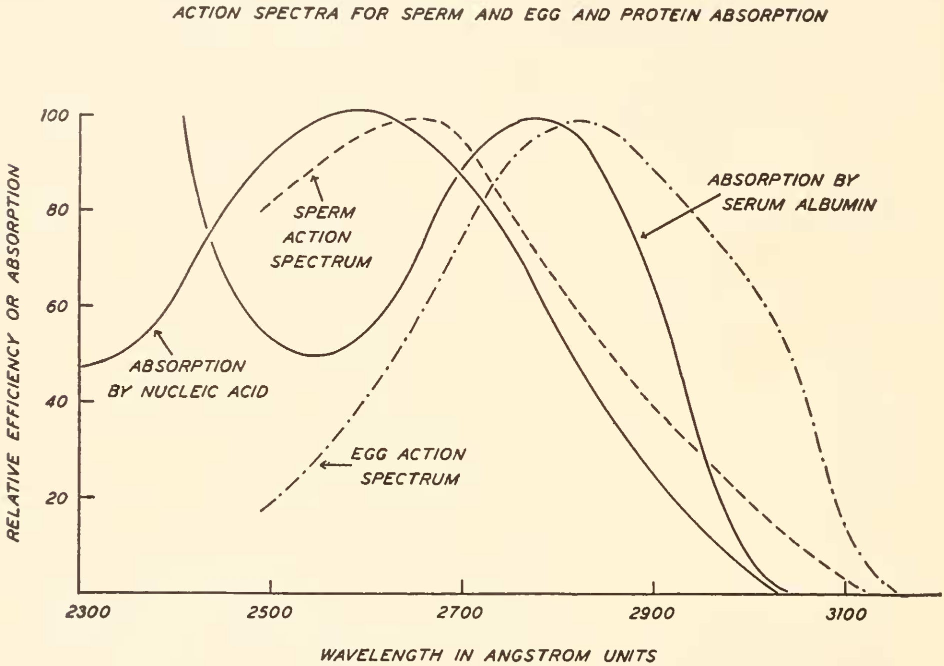

FIGURE 2. Comparison of the action spectra of Figure 1 with absorption spectra of nucleic acid and serum albumin. Data for nuclei acid from Caspersson (1938), for proteins from Smith (1929). Note that the action spectrum for the egg is quite different from the absorption spectrum for albumin at both ends.

nucleus are involved the egg may show an action spectrum different from that of the sperm.

The methods employed for the studies at different wave-lengths are similar to those already described elsewhere (Giese, 1938, 1939c), therefore, only the briefest mention need be made of them. The irradiated eggs are fertilized with normal sperm. The rate of division is then determined by observing for percentage of cleavage every 15 minutes. The times at which the eggs reach the 2, 4, 8, 16, and 32 cell stages are recorded and the number of cleavages is plotted against the time after fertilization and compared with the control. The increase in time required to reach the third cleavage is taken as a measure of the retarding action of the radiations. The retardation is then plotted against dosage. From such curves for each of the wave-lengths the dosage required to bring about a given retardation can be determined. For Figures 1 and 2 the reciprocals of the relative amounts of energy at different wave-lengths required to produce a retardation of division by 1.5 hours were determined. In Figure 1 at *A* and *C* the sperm and egg are compared on this basis and a great difference in susceptibility between the gametes is evident. In *B* the data for the eggs are compared amongst themselves on a relative basis setting the action at λ 2,804 Å as 100 per cent efficient.

The shape of the curves indicates that different materials are being affected in the two cases, since the action spectrum is considered to be a measure of the absorption by the active constituent. To see if the absorbing materials can be identified the absorption spectra for serum albumin and nucleic acid are given in Figure 2. It is apparent that the action spectrum for sperm matches the absorption spectrum for nucleic acid better than the absorption spectrum for albumin; the reverse is true for the action spectrum of the egg. Since the simple proteins and nucleoproteins are the major structural constituents of the cell and none of the other organic or inorganic constituents have very specific absorption, the resemblances while imperfect are indicative of absorption by these two classes of compounds in the action of ultraviolet radiations on the gametes.

Discussion

The occurrence of a differential susceptibility of gametes with the sperm more sensitive to ultraviolet light than the egg as first found in the sea urchin, *Strongylocentrotus purpuratus,* was verified on Arbacia and Dendraster and in preliminary trials on Pateria and *S. franciscanus* but not on Urechis, Chaetopterus, and Nereis. In the latter forms the sperm appears to be only slightly more sensitive than the egg (Table I). The former group of species belongs embryologically to the radially-cleaving, indeterminate egg type, the latter group to the spiral determinate type. In addition, the radial eggs used here are mature or nearly so at the time of shedding whereas the spiral eggs are generally immature. An illustration of the difference in response to ultraviolet light, depending on this difference in organization is seen in the local "burns" occurring in the spiral eggs. Thus a Nereis egg given a unilateral dosage of between 8,000–16,000 ergs/mm.^{2} may develop apparently normally except on the burned surface which appears blistered. A Strongylocentrotus egg on the other hand unless given a large dosage of light will show general effects distributed throughout the retarded egg. However, it is not possible to say which features of the organization account for the difference in sensitivity of the eggs and sperm of the two groups.

One might envisage that in eggs the retarding effects of radiation on cleavage are due to the inactivation, by substances formed during irradiation, of some catalyst which is necessary for the reactions involved in cleavage. In one group of eggs perhaps the catalyst is present in excess of that necessary for a characteristic rate of cleavage, the rate being controlled by some other limiting factor, in the other it is present in just adequate concentration and itself constitutes the limiting factor. Even a considerable dosage of radiations will not reduce the concentration of catalyst below the critical level in the first case but will readily do so in the second. In the first case no cleavage delay would be expected until very large dosages of radiations had been administered, in the second the cleavage should be affected after very small dosages. One would have to assume that irradiated sperm on penetrating unirradiated eggs introduce similar cleavage-inhibiting substances acting on the catalyst as those formed in the irradiated egg. In this case also the effect on cleavage should depend upon the amount of catalyst present in the egg—if in excess, the cleavage should not be easily inhibited, if limiting, the reverse should be true. We should expect both sperm and egg to be relatively insensitive to the radiations in the former and this is found in most spirally cleaving eggs.

Against the above postulation is the fact that the action spectrum for sperm resembles nucleoprotein absorption while for the egg it resembles simple protein absorption indicating two different ultraviolet absorbing materials in the gametes by which the cleavage-retarding effect is produced. It is possible that absorption by both of these types of proteins leads to the formation of toxic photoproducts which inhibit the same catalyst. It is also possible that the toxic substance is much more rapidly formed by the nucleoproteins, but the necessary assumptions strain the imagination.

It should be pointed out that the retardation of the early cleavage is only the initial effect of the radiation. If the delayed effect could be studied we might find that the recovery from injury to the egg would resemble absorption by nucleoprotein, indicating a more lasting injury to the nucleus than to the cytoplasm, as is the case for division of Paramecium (Giese, 1945a). Because the number of cells cannot be satisfactorily determined in the later cleavages such experiments were not attempted with eggs.

The action spectrum obtained for the egg is similar to that observed for "cytoplasmic" effects such as increased time of ciliary reversal, retardation of excystment, immobilization of cilia, and prevention of hatching of eggs. The action spectrum for the sperm resembles that for "nuclear" effects such as recovery of paramecia from sublethal effects, bactericidal and fungicidal effects and the production of mutations (see Giese, 1945b, for references). It is interesting to note the difference between the action spectrum for retardation of cleavage of the egg and for activation studied by Hollaender (1938). In the latter case no action was found until about λ 2,650 Å and the effectiveness of the light increased as the wave-length decreased. The mechanism of action of the light must be different in these two instances. The action spectrum data thus lay the foundation for further analysis of the effect of these radiations upon gametes.

Summary

1. The action spectrum for the retardation of division of eggs fertilized with irradiated sperm resembles the absorption of ultraviolet light by nucleoproteins.

2. The action spectrum for retardation of division of irradiated eggs of the sea urchin resembles absorption by simple proteins like albumin except that at the short wave-length end there is no increase in action at λ 2,483 Å where absorption shows a definite upswing.

3. The absolute amount of energy required to affect division to the same extent by affecting the sperm is very much less than that required to affect eggs.

4. Other Echinoderms tested show a similar difference in susceptibility of eggs and sperm: *S. franciscanus, Arbacia punctulata, Dendraster excentricus,* and *Pateria miniata*

5. Animals other than Echinoderms tested did not show as striking a difference between susceptibility of eggs and sperm: *Urechis caupo, Mactra* sp., *Chaetopterus pergamentaceus,* and *Nereis limbata.*

6. In the eggs listed in paragraph 5, determinations are made more difficult by the tendency for the eggs to show irregular cleavage rather than retarded cleavage as the dosage increases. Such irregular cleavage occurs in Echinoderm eggs as well but the threshold is higher.

7. If both eggs and sperm of the sea urchin are irradiated the effect on the rate of division is less than the sum of the effects which would be expected on each of the gametes alone. However, the percentage of abnormal cleavage greatly increases.

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